CLAIMS

We claim:

1. An isolated recombinant human metapneumovirus (rHMPV), comprising

a partial or complete, recombinant HMPV genome or antigenome comprising one or more attenuating nucleotide modifications, and

a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), and a large polymerase protein (L).

2. The rHMPV of claim 1, wherein the rHMPV is replication competent.

3. The rHMPV of claim 1, wherein the recombinant HMPV genome or antigenome further comprises a detectable heterologous sequence encoding a polypeptide.

4. The rHMPV of claim 3, wherein the detectable heterologous sequence encodes a reporter.

5. The rHMPV of claim 4, wherein the reporter comprises green fluorescent protein (GFP).

6. The rHMPV of claim 3, wherein the detectable heterologous sequence is operably linked to HMPV transcription gene start and gene end signals.

7. The rHMPV of claim 1, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of one or more rHMPV SH, G, M2-1, M2-2, or M2 ORFs or one or more nucleotide substitutions that reduces or ablates expression of the one or more rHMPV SH, G, M2-1, M2-2, or M2 ORFs.

8. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of one or more of the rHMPV SH, G, M2-1, M2-2, or M2 ORFs, such that a functional protein is not produced.

9. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a rHMPV SH ORF, such that a wild type SH protein is not produced.

10. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a SH ORF of SEQ ID NO: 1, such that a SH protein comprising a sequence set forth as SEQ ID NO: 5 is not produced.

11. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a rHMPV G ORF, such that a wild type G protein is not produced.

12. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a G ORF of SEQ ID NO: 1, such that a G protein comprising a sequence set forth as SEQ ID NO: 6 is not produced.

13. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a rHMPV SH and G ORFs, such that a wild type SH protein and a wild type G protein are not produced.

14. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a SH ORF and a G ORF of SEQ ID NO: 1.

15. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises one or more nucleotide substitutions that reduces or ablates expression of a rHMPV M2-2 ORF.

16. The rHMPV of claim 15, wherein the one or more nucleotide substitutions that reduces or ablates expression of the rHMPV M2-2 ORF comprises one or more nucleotide substitutions that ablates one or more potential translation initiation codons of the rHMPV M2-2 ORF or introduces one or more in-frame stop codons into the r HMPV M2-2 ORF.

17. The rHMPV of claim 16, wherein the one or more nucleotide substitutions comprises substitutions of one or more nucleic acids of a rHMPV sequence set forth as SEQ ID NO: 1.

18. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a rHMPV M2-2 ORF, such that a wild type M2-2 protein is not produced.

19. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a M2-2 ORF of SEQ ID NO: 1.

20. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises one or more nucleotide substitutions that reduces or ablates expression of a rHMPV M2-1 ORF, such that a wild type M2-1 protein is not produced.

21. The rHMPV of claim 20, wherein the one or more nucleotide substitutions that reduces or ablates expression of the rHMPV M2-1 ORF comprises one or more nucleotide substitutions that ablates the translation initiation codon of the rHMPV M2-1 ORF and further ablates additional ATG triplets in each reading frame of the rHMPV M2-1 ORF.

22. The rHMPV of claim 21, wherein the one or more nucleotide substitutions comprises substitutions at one or more positions of SEQ ID NO: 1.

23. The rHMPV of claim 7, wherein the one or more attenuating nucleotide modifications comprises a partial or complete deletion of a rHMPV M2 ORF.

24. The rHMPV of claim 23, wherein the partial or complete deletion comprises a partial or complete deletion of the M2 ORF of SEQ ID NO: 1.

25. The rHMPV of claim 1, wherein the one or more attenuating nucleotide modifications produces at least one desired phenotypic change in the rHMPV, wherein the phenotypic change comprises at least one of a change in growth properties in cell culture, a change in growth properties or virulence in the upper or lower respiratory tract of a mammalian host, a change in viral plaque size, a change in sensitivity or adaptation to temperature, a change in cytopathic effect, a change in the efficiency of transcription or genome replication, a change in sensitivity to interferon, a change in the efficiency of expression of one or more genes, or a change in immunogenicity.

26. The rHMPV of claim 25, wherein the one or more attenuating nucleotide modifications produces a change in viral growth in the upper respiratory tract, lower respiratory tract, or both, such that viral growth is attenuated by about 50-100 fold or greater, compared to growth of the corresponding wild type HMPV strain.

27. The rHMPV of claim 1, wherein the one or more attenuating nucleotide modifications comprises one or more nucleotide substitutions that produce one or more amino acid substitutions in a M2-1 or a L protein in the rHMPV.

28. An isolated, replication competent recombinant human metapneumovirus (rHMPV), comprising a partial or complete, recombinant HMPV genome or antigenome, a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), and a large polymerase protein (L), wherein the genome or antigenome of the rHMPV is rearranged such that an order of one or more genes or genome segments in the recombinant HMPV genome or antigenome is altered as compared to a wild type HMPV.

29. The rHMPV of claim 28, wherein the order of a SH, G, or F gene or genome segment is altered in the rHMPV genome or antigenome.

30. The rHMPV of claim 28, wherein the rHMPV genome or antigenome comprises a SH gene or genome segment and a G gene or genome segment inserted after a M gene and before a F gene of the rHMPV genome or antigenome.

31. The rHMPV of claim 28, wherein the rHMPV genome or antigenome comprises at least two copies of a SH gene or genome segment and at least two copies of a G gene or genome segment inserted after a M gene and before a F gene of the rHMPV genome or antigenome.

32. The rHMPV of claim 28, wherein the rHMPV genome or antigenome comprises a F gene or genome segment inserted after a 3' leader sequence and before a N gene of the rHMPV genome or antigenome.

33. The rHMPV of claim 28, wherein the rHMPV genome or antigenome comprises a G gene or genome segment inserted after a 3' leader sequence and before a N gene of the rHMPV genome or antigenome.

34. The rHMPV of claim 28, wherein the rHMPV genome or antigenome comprises a F gene or genome segment and a G gene or genome segment inserted after a 3' leader sequence and before a N gene of the rHMPV genome or antigenome.

35. The rHMPV of claim 28, wherein the rHMPV genome or antigenome comprises a G gene or genome segment and a F gene or genome segment inserted after a 3' leader sequence and before a N gene of the rHMPV genome or antigenome.

36. The rHMPV of claim 1, wherein the one or more attenuating nucleotide modifications comprises inserting one or more additional copies of one or more rHMPV G or F genes or genome segments in the rHMPV genome or antigenome.

37. The rHMPV of claim 36, wherein the rHMPV genome or antigenome comprises one or more additional copies of a rHMPV G gene or genome segment, a F gene or genome segment, or both, inserted after a 3' leader sequence and before a N gene of the rHMPV genome or antigenome.

38. The rHMPV of claim 36, wherein the rHMPV genome or antigenome comprises a single additional copy of a rHMPV G gene or genome segment inserted after a 3' leader sequence and before a N gene of the rHMPV genome or antigenome.

39. The rHMPV of claim 36, wherein the rHMPV genome or antigenome comprises a single additional copy of a rHMPV F gene or genome segment inserted after a 3' leader sequence and before a N gene of the rHMPV genome or antigenome.

40. The rHMPV of claim 36, wherein the rHMPV genome or antigenome comprises one additional copy of the rHMPV G gene and one additional copy of the rHMPV F gene in the order G-F.

41. The rHMPV of claim 36, wherein the rHMPV genome or antigenome comprises one additional copy of the recombinant HMPV G gene and one additional copy of the recombinant HMPV F gene in the order F-G.

42. The rHMPV of claim 36, wherein the rHMPV genome or antigenome comprises one additional copy of a rHMPV G gene or genome segment and two additional copies of the rHMPV F gene or genome segment in the order G-F-F.

43. The rHMPV of claim 1, wherein the rHMPV genome or antigenome further comprises one or more heterologous genes or genome segments from a different paramyxovirus to form a chimeric recombinant HMPV genome or antigenome.

44. The rHMPV of claim 43, wherein the rHMPV genome or antigenome comprises one or more N, P, or M genes from a different paramyxovirus.

45. The rHMPV of claim 44, wherein the paramyxovirus comprises avian metapneumovirus.

50. The rHMPV of claim 1, wherein the rHMPV genome or antigenome further comprises one or more rHMPV genes or genome segments from a different subgroup of HMPV to form a chimeric recombinant HMPV genome or antigenome.

51. An immunogenic composition comprising an immunogenically effective amount of any one of the isolated, replication competent recombinant human metapneumoviruses of claims 1-50 in a pharmaceutically acceptable carrier.

52. A method for inducing an immune response in a subject against human metapneumovirus, comprising administering to the subject a therapeutically effective amount of any one of the isolated, replication competent recombinant human metapneumoviruses of claims 1-50, thereby inducing an immune response in the subject against human metapneumovirus.

53. The method of claim 52, wherein the recombinant human metapneumovirus is administered in a dose of 10^3 to 10^7 PFU.

54. The method of claim 53, wherein the recombinant human metapneumovirus is administered to the upper respiratory tract.

55. The method of claim 52, wherein the recombinant human metapneumovirus is administered by spray, droplet or aerosol.

56. An isolated, replication competent recombinant virus comprising a paramyxovirus genome or antigenome and a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and one or more recombinant genes or genome segments from human metapneumovirus.

57. The recombinant virus of claim 56, comprising a human metapneumovirus F gene.

58. The recombinant virus of claim 56, wherein the virus is an influenza virus or a parainfluenza virus.

59. An expression vector comprising an operably linked transcriptional promoter, a partial or complete, recombinant human metapneumovirus (rHMPV) genome or antigenome, and a transcriptional terminator.

60. The expression vector of claim 59, wherein the rHMPV genome or antigenome comprises one or more attenuating nucleotide modifications.

61. A method of screening an antiviral compound for inhibition of a biological activity of a human metapneumovirus, comprising

providing a recombinant human metapneumovirus (rHMPV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a partial or complete, recombinant HMPV genome or antigenome modified to incorporate a detectable heterologous sequence encoding a polypeptide correlated with the biological activity upon expression of the heterologous sequence;

exposing a test sample comprising the rHMPV or a host cell amenable to infection by HMPV to a test compound or library of test compounds that prospectively includes one or more antiviral agents capable of inhibiting the biological activity of HMPV;

providing a control sample comprising the rHMPV or host cell under suitable control conditions in the absence of the test compound or library of test compounds; and

detecting heterologous sequence in the test and control samples to determine an increase or decrease of the biological activity in the test sample compared to the control sample to determine the presence or absence of the antiviral compound in the test sample.